Applicant : Yann Echemateral

Serial No.: 09/884,586 Filed: June 19, 2001

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Attorney & Docket No.: 10275-120001 / TCI-120

A1 COO2 human PDGF A chain, an IRES and a nucleic acid sequence encoding human PDGF B chain. This 2 kb insert was ligated into the mammary gland expression vector pBC450 (nucleic acid sequence provided; SEQ ID NO:2), to create the expression cassette pBC734. The nucleic acid sequence of the PDGF-B insert of expression vector pBC701 is also provided (SEQ ID NO:1). This insert was ligated into the mammary gland expression vector pBC450 (nucleic acid sequence provided), to create the expression cassette pBC701.--

Replace the paragraph beginning at page 36, line 10, with the following rewritten paragraph:

-- To create BC701, the vector pSBC-PDGF-A/-G-B was first cut partially with restriction enzyme HindIII and was ligated to the self-annealing cohesive linker HINXHO (sequence: AGCTCTCGAG; SEQ ID NO:4). Integration of this linker destroys the HindIII site and creates and Xho I site in its place. The plasmid pAB21 which had one copy of HINXHO integrated in the HindIII site located at the 3' end of the PDGF-B gene was identified using restriction enzyme mapping. Plasmid pAB21 was then partially cut with the restriction enzyme Eco RI and was ligated to the self-annealing cohesive linker ECOXHO (sequence: AATTCTCGAG; SEQ ID NO:5). Integration of this linker into an EcoRI site creates a Xho I site. The plasmid pAB23 which had one copy of ECOXHO integrated in the EcoRI site located just at the 5' end of the PDGF-B gene was identified using restriction enzyme mapping. Complete digestion of pAB23 with the restriction enzyme XhoI liberates an approximately 750 bp fragment containing the full sequence of the PDGF-B190 gene. PDGF-B190 is a specific gene construct described in detail in EP 658 198. It codes for a translation product (PDGF-BB), which is identical to fully processed mature PDGF-BB. In the construct a stop codon was introduced in position 191 of the PDGF-B precursor protein. As a result, the carboxy-terminal part of the PDGF-B molecule, which is responsible for the retention of incompletely processed forms, is not expressed.--

Replace the paragraph beginning at page 37, line 4, with the following rewritten paragraph:

--To create the PDGF-A-IRESG-PDGF-B expression cassette, the intermediate vector pAB21 was first digested to completion with the restriction enzyme NotI. The ends were filled with



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Klenow DNA polymerase and the resulting fragment was self-ligated. In the resulting plasmid, pAB2, the restriction site NotI located in the IRES/G sequence had been destroyed. The intermediate vector pAB2 was then cut partially with the restriction enzyme Eco RI and was ligated to the self-annealing cohesive linker ECONOXHO (sequence: AATTGCTCGAGC; SEQ ID NO:6). Integration of this linker into an EcoRI site creates and Xho I site while destroying the EcoRI site. The plasmid pAB33 which had one copy of ECONOXHO integrated in the EcoRI site located just at the 5' end of the PDGF-A gene was identified using restriction enzyme mapping. Complete digestion of pAB33 with the restriction enzyme XhoI liberates an approximately 2 kb fragment containing the full sequence of the PDGF-A gene as well as the full sequence of the PDGF-B190 gene; both genes were separated by the IRESG sequences. This 2 kb fragment was isolated and ligated into the mammary gland expression vector pBC450, to create the expression cassette pBC734 (Figure 1).--

Replace the paragraph beginning at page 38, line 31, with the following rewritten paragraph:

-- The following primers were used:

GBC 332: TGTGCTCCTCTCCATGCTGG(SEQ ID NO:7)

GBC 386: TGGTCTGGGGTGACACATGT(SEQ ID NO:8)--

In the drawings:

Substitute the enclosed 16 sheets of formal drawings for the informal drawings as filed.